

FRDA REPORT 113

IDENTIFICATION OF KEY PATHOGENS
OF MAJOR COASTAL FOREST WEEDS

Thomas E. [redacted] er
Francesca Sielma-Canavesi
Charles E. Dorworth

Departmental Representatives:

Charles E. Dorworth
Forestry Canada
Pacific and Yukon Region
Development Branch
508 W. Burnside Road
Victoria, B.C.
V8Z 1M5

March, 1990

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ISSN 0835-0752
ISBN 0-662-17714-2

Canadian Cataloguing in Publication Data

Sieber, Thomas E.

Identification of key pathogens of major coastal forest weeds.

(FRDA report, ISSN 0835-0752; 113)

On cover: Canada/BC Forest Resource Development Agreement

Includes bibliographical references.

ISBN 0-662-17714-2

1. *Acer macrophyllum* - Biological control - British Columbia - Vancouver Island. 2. Red alder - Biological control - British Columbia - Vancouver Island. 3. Fungi, Pathogenic - British Columbia - Vancouver Island. 4. Conifers - Weed control - British Columbia - Vancouver Island. I. Sieber - Canavesi, Francesca. II. Dorworth, C. E. III. Canada. Forestry Canada IV. Canada - British Columbia Forest Resource Development Agreement. V. Title. VI. Series.

SB608.M34S53 1990 634.9'72264 C90-092103-X

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This study was funded by the Swiss National Research Council (grant no. 823A-025103) and Forestry Canada under the Canada-British Columbia Forest Resource Development Agreement (1985-1990). This report has been reviewed by Forestry Canada and approved for distribution. This approval does not necessarily signify that the contents reflect the views or policies of Forestry Canada.

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Abstract

Bigleaf maple (Acer macrophyllum Pursh) and red alder (Alnus rubra Bong.) are weeds in the Coast Forest Region of British Columbia, Canada, when they grow to the detriment of commercially valuable conifers. Research carried out at Pacific Forestry Centre, Forestry Canada, is directed toward biological control of bigleaf maple and red alder. The selection of candidate bioagents (mycoherbicides) has been limited by our choice to native or indigenous microorganisms.

So far, search for candidates was restricted to isolations of fungi from diseased plant tissues. This study was carried out in order to prove the possibility in finding some potentially pathogenic fungi within the population of endophytic fungi.

Apparently healthy leaves and 2- to 3-yr-old twigs of both bigleaf maple and red alder were collected at seven and eight sites, respectively, surface-sterilized, cut into small pieces and incubated on malt extract agar. The species most frequently isolated were Cryptodiaporthe hystrix, Diaporthe eres, Pezicula livida, Phomopsis sp. 1, and Phomopsis sp. 2 on bigleaf maple as well as Gnomonia setacea, Gnomoniella tubaeformis, Ophiovalsa suffusa, and Phomopsis sp. 2 on red alder.

The frequency of colonization was always strongly dependent on the site. Correlations with weather conditions, however, existed only for Melanconis alni and the overall colonization of red alder: the more snow the more M. alni and the more endophytic fungi.

Three potentially pathogenic endophytes have been found on bigleaf

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maple: Cryptodiaporthe hystrix, Diaporthe eres, and Glomerella cinquilata. Six pathogenic species have been isolated from red alder: Diaporthe eres, Gnomonia setacea, Gnomoniella tubaeformis, Melanconis alni, Nectria sp., and Septoria alni. These species were discussed in respect to their frequency of occurrence as endophytes, their morphology, as well as their pathogenicity and were used to calculate the Latent Disease Potential (LDP) at each site.

Introduction

Bigleaf maple (Acer macrophyllum Pursh) and red alder (Alnus rubra Bong.) are tree species of the Coast Forest Region of British Columbia. They commonly grow in pure stands as early occupants on cleared land. As two of the first colonizers of clear-cut and burned areas, red alder and bigleaf maple are considered to be weeds because they grow faster than the commercially more valuable conifers that are usually planted.

Chemical herbicides and controlled burning are silvicultural tools that often permit adequate weed control after logging. Use of either burning or spraying of chemical herbicides in forest renewal efforts is becoming more and more difficult in some instances because of public reaction.

The Pacific Forestry Centre (PFC) has begun research to develop essential biological control strategies in order to provide an

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alternative vegetation management tool for use in environmentally sensitive areas in particular and others when appropriate (Dorworth 1986). The selection of biological control agents was limited as a matter of policy to native or indigenous microorganisms, and, of course, successful candidates should only affect the weed species and not damage the crop itself.

Microorganisms which fit these conditions best can be found on the weed species itself. Although microorganisms with the desired pathogenic properties include bacteria, viruses and obligate biotrophic fungi, research is mainly directed towards the development of mycoherbicides based on facultative biotrophic fungi (i. e. fungi able to grow on both living plant tissues and dead substrates (saprophytically)). The pathogenicity of these fungi is usually low by nature because they have co-evolved with their respective host. Therefore, the host's defense mechanisms are very well developed. In order to shift the balance of the pathogenic interaction in favour of the fungus the PFC enhancement process has been developed (Dorworth 1988, 1989).

So far, search for mycoherbicide candidates was limited to isolations of fungi from diseased plant material. This investigation intends to prove the usefulness of the examination of symptomless specimens as a means to identify and culture endophytic fungi which are potential candidate mycoherbicides. Indications in the literature as well as our own research work confirm that most of the pathogenic fungi have a latency period during which they live endophytically in healthy plant tissues (Canavesi 1983, Stone 1987, Carroll 1988, Sieber 1988, Sieber et al. 1988, Sieber et al. 1989). The duration of

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this period can be some days to some years depending on the pathogen as well as on climatical and ecological conditions. Thus, it can be expected that most of the fungi producing disease symptoms can be detected as endophytes in healthy tissues long before symptoms become visible.

Another advantage of the isolation of endophytic fungi is the possibility to sample ecological data about the number of fungal species, their distribution and frequency as well as their interactions. These statistics are a measure of the latent disease potential inherent in a healthy plant individual or plant stand and could be used to select and quantify the appropriate means of disease enhancement or, where it is desirable to protect such plants as a crop, the appropriate means of protection.

Materials and Methods

Definition of endophytic fungi

'Endophytic' or 'endophyte' (from Greek 'endo' = inside, 'phytos' = plant) are, strictly speaking, positional expressions indicating that the organism is located inside plant tissue regardless of the health of the tissue, but the use of these expressions is usually restricted to organisms living inside healthy plant tissues at the moment of detection. In these studies, 'endophyte' is used in the latter sense.

Experimental design

Leaves and 2- to 3-yr-old twigs of healthy 5- to 10-yr-old red alders and bigleaf maples were collected at eight and seven sites, respectively, in British Columbia during fall 1988 (Fig. 1). Ten trees per species were cut on each site. Ten apparently healthy twigs were excised at random from each tree and, from sites 1, 2 and 3, ten healthy leaves were also removed. All tissues were processed within 24 hours after collection using the following method.

Method of isolation

The plant material was dipped in 95% ethanol for 60 seconds, and surface-sterilized in 4% sodium-hypochlorite for 5 minutes, followed by washing for 30 seconds in 95% ethanol (Sieber 1989). Then, a piece of tissue with an area of approximately one-quarter squared centimetre was removed from the middle of each leaf, and a disk of 1 to 2 millimetre thickness was cut from the middle of each twig and plated out on 2 % malt extract agar supplemented with 8 ppm Novo-Tetramycin in order to avoid bacterial growth. After 2 to 8 weeks incubation at 20°C in darkness, the endophytic fungi grew out of the twig and leaf pieces. Each individual thallus was cultivated in pure culture until fructifications allowed identification.

Meteorological data

Table 1 shows the summarized monthly records (provided by the Atmospheric Environment Service of Environment Canada) of the meteorological stations closest to the collection sites (Fig. 1) between March 1985 and August 1988. These data reflect exactly the

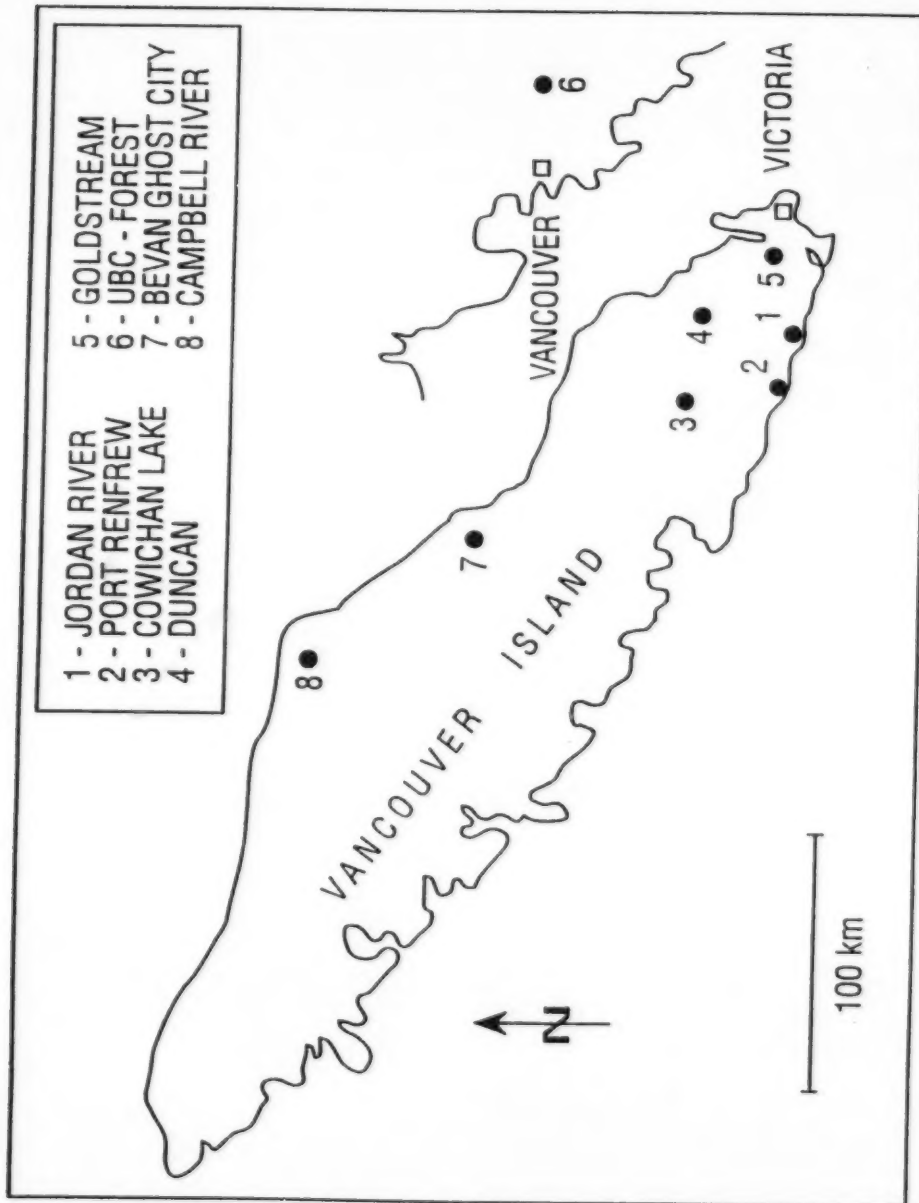


Fig. 1: Sites on which collections of red alder and bigleaf maple were made to examine for presence of endophytic fungi.

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weather conditions during the life of the examined 3-yr-old twigs.

Table 1: Weather conditions at the collection sites between March 1985 and August 1988.

Site # Name	Sum of the monthly records between March 1985 - August 1988:			Temperature [°C] ¹	
	Rain [mm]	Snow [mm]	Number of days with rain ²	frost ³	
1 Jordan River	7111	98	609	170	13.4
2 Port Renfrew	10578	109	707	142	12.5
3 Cowichan Lake	6003	248	576	166	14.0
4 Duncan	3046	163	483	231	14.3
5 UBC-Forest	6475	106	579	174	14.3
6 Gold Stream	3244	255	530	167	14.2
7 Bevan Ghost City	4320	126	580	151	14.0
8 Campell River	4167	171	618	285	13.5

Morphological studies

The descriptions of cultures are based on fungal growth on 2 % malt extract agar (MEA). Variations in size were evaluated by measuring 10 each of randomly selected spores, conidia, conidiophores etc..

Statistical data analysis

Frequencies of colonization are given as percentage of leaves or twigs colonized by endophytic fungi (number of trees, twigs, or leaves examined = 100 %).

. Average temperature during the vegetation periods (April to September) 1985 - 1988

. Days with more than 0.2 mm rain

. Days with at least one measurment < 0°C

In order to compare the species composition of the endophyte populations of the twigs and the leaves similarity indices were calculated:

$$\lambda = \frac{2 \cdot z}{x + y}; \quad 0 \leq \lambda \leq 1;$$

λ = similarity index;

x = number of species occurring in the leaves;

y = number of species occurring in the twigs;

z = number of species occurring in both organs.

Frequencies of colonization were examined for significant differences using the Mann-Whitney U test (Riedwyl 1978) and displayed graphically as box plots (Cleveland 1985).

Regression analysis was used to measure the extent of correlation between frequencies of colonization and meteorological conditions.

All calculations were effected using a programmable TI-66 (Texas Instruments).

Definition of the 'Latent Disease Potential' (LDP)

In order to estimate the latent disease potential inherent in a healthy red alder or bigleaf maple stand the 'Latent Diseases Potential' (LDP) was defined. The LDP is based on the number of trees and twigs/leaves colonized by endophytic fungi which are known to be pathogenic. It is calculated as follows:

$$LDP = \sum_{i=1}^n p_i \sqrt{a_i^2 + b_i^2};$$

a = number of trees colonized;

b = number of twigs/leaves colonized;

p = pathogenicity of endophyte i;

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i = endophyte species number i ($i = 1, 2, 3, \dots, n$);
 n = number of endophytic species with pathogenic properties.

Geometrically, LDP is a sum of vector lengths. The two components of each vector are the number of trees and the number of twigs/leaves colonized by a pathogenic endophyte species.

The factor p_i is the estimated pathogenicity of a species in relation to the pathogenicity of each other species under consideration. The range of p is arbitrary: if $1 \leq p \leq 10$, for example, $p=10$ is given to the endophyte with the strongest and $p=1$ to the one with the weakest pathogenicity. In this investigation p was chosen to be constant [$p = 1$] for all the pathogenic endophytes.

Results and Discussion

BIGLEAF MAPLE

Overall colonization

Eighty-three percent of the leaves and almost 52 % of the twigs were colonized by at least one endophyte (Table 2). The frequency of twigs colonized by two different species was low, whereas that of leaves colonized by two species was almost 20 %.

Table 2: Frequency of colonization of leaves and twigs of bigleaf maple by endophytic fungi.

<u>Taxon</u>	i.c. ⁴	frequency (%) of	
		leaves	twigs
Number of leaves/twigs examined	200	670	
Percentage of leaves/twigs colonized by endophytic fungi	83.0	51.5	
-colonized by one species	63.0	46.7	
-colonized by two species	19.0	4.5	
-colonized by three species	1.0	0.3	
Ascomycetes			
<u>Coniochaeta</u> sp., anamorph: <u>Phialophora</u>	a+t ⁵	0	0.1
<u>Cryptodiaporthe hystrix</u> (Tode) Petrak, anam.: <u>Diplodina acerina</u> (Pass.) Sutton	a	2.0	28.4
<u>Diaporthe eres</u> Nitschke, anam.: <u>Phomopsis occulta</u> (Sacc.) Trav.	a	20.0	3.0
<u>Glomerella cingulata</u> (Stonem.) Spauld. & v. Schrenk., anam.: <u>Colletotrichum</u> <u>gloeosporioides</u> (Penz.) Penz. & Sacc.	a+t	3.0	1.8

. i.c. = in culture: a = anamorph formed, t = teleomorph formed, s = sterile
 . only sterile ascomata formed

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Table 2 continued

<u>Taxon</u>		frequency (%) of	
		i.c. colonization of	leaves twigs
<u>Hypoxylon</u> <u>serpens</u> (Pers.: Fr.) Kickx, anam.: <u>Geniculosporium</u>	a	2.0	0.7
<u>Mycosphaerella</u> <u>punctiformis</u> (Pers.: Fr.) Schroet., anam.: <u>Ramularia</u>	a	3.0	0
<u>Pezicula</u> <u>livida</u> (Berk. & Br.) Rehm, anam.: <u>Cryptosporiopsis</u> <u>abietina</u> Petrak	a	1.0	5.2
<u>Xylaria</u> sp.	s	1.0	0.9
Deuteromycetes			
Coelomycetes			
<u>Coniothyrium</u> sp.		0	0.1
<u>Cryptosporiopsis</u> sp.		2.0	0.7
<u>Cytospora</u> sp.		1.0	0
<u>Phomopsis</u> sp. 1		28.0	5.2
<u>Phomopsis</u> sp. 2		18.0	2.1
<u>Phomopsis</u> sp. 3		0.5	0
<u>Phomopsis</u> sp. 4		0.5	0
<u>Stigmina</u> sp.		0	0.1
Hyphomycetes			
<u>Aureobasidium</u> <u>pullulans</u> (de Bary) Arn.		0	0.3
<u>Cladosporium</u> <u>cladosporioides</u> (Fresen.) de Vries		1.0	0.3
<u>Cladosporium</u> <u>sphaerospermum</u> Penz.		0	0.1
<u>Epicoccum</u> <u>purpurascens</u> Ehrenb.: Schlecht.		1.0	0
<u>Monodictys</u> <u>paradoxa</u> (Corda) Hughes		0	0.1
<u>Periconia</u> <u>byssoides</u> Pers.: Méral		0	0.1
<u>Phialophora</u> <u>hoffmannii</u> (Beyma) Scholl-Schwarz		0.5	0.1
Sterile Mycelia		19.5	7.3

Depending on the site the overall colonization varied between 76 (Port Renfrew) and 94 % (Jordan River) for leaves as well as between 19 (Bevan Ghost City) and 71 % (Port Renfrew) for twigs.

The sites were not significantly different in the overall colonization of leaves (Mann Whitney U test, $p < 0.05$). At Bevan Ghost City, however, the overall colonization of twigs was

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significantly lower than that at any other site (Fig. 2). The among-tree variation of the overall colonization of twigs was highest at UBC-Forest (20-90 %) and smallest at Bevan Ghost City (10-30 %).

Similarity of mycoflora within leaves and twigs

According to Table 2 the similarity index is 0.72 (only species with frequency ≥ 0.5 % included in calculation). All the species (≥ 0.5 %) isolated from the twigs were found on the leaves, too. This implies that the endophyte populations of twigs and leaves were similar in respect to their species compositions. But in respect to the frequencies of most of these species the two organs were quite different.

Colonization by frequently isolated and known pathogenic species

The endophyte population of the leaves was dominated by Diaporthe eres, Phomopsis sp. 1 and Phomopsis sp. 2 which occurred in more than 10 % of the leaves. Cryptodiaporthe hystrix was the only species occurring in more than 10 % of the twigs followed by Pezicula livida and Phomopsis sp. 1 which occurred in 5.2 % of the twigs (Table 2). Three potentially pathogenic species could be isolated from both the leaves and the twigs: C. hystrix, D. eres, and Glomerella cinquilata. These species are described and discussed below in respect to their morphology, taxonomy, pathogenicity, distribution and frequency as endophytes:

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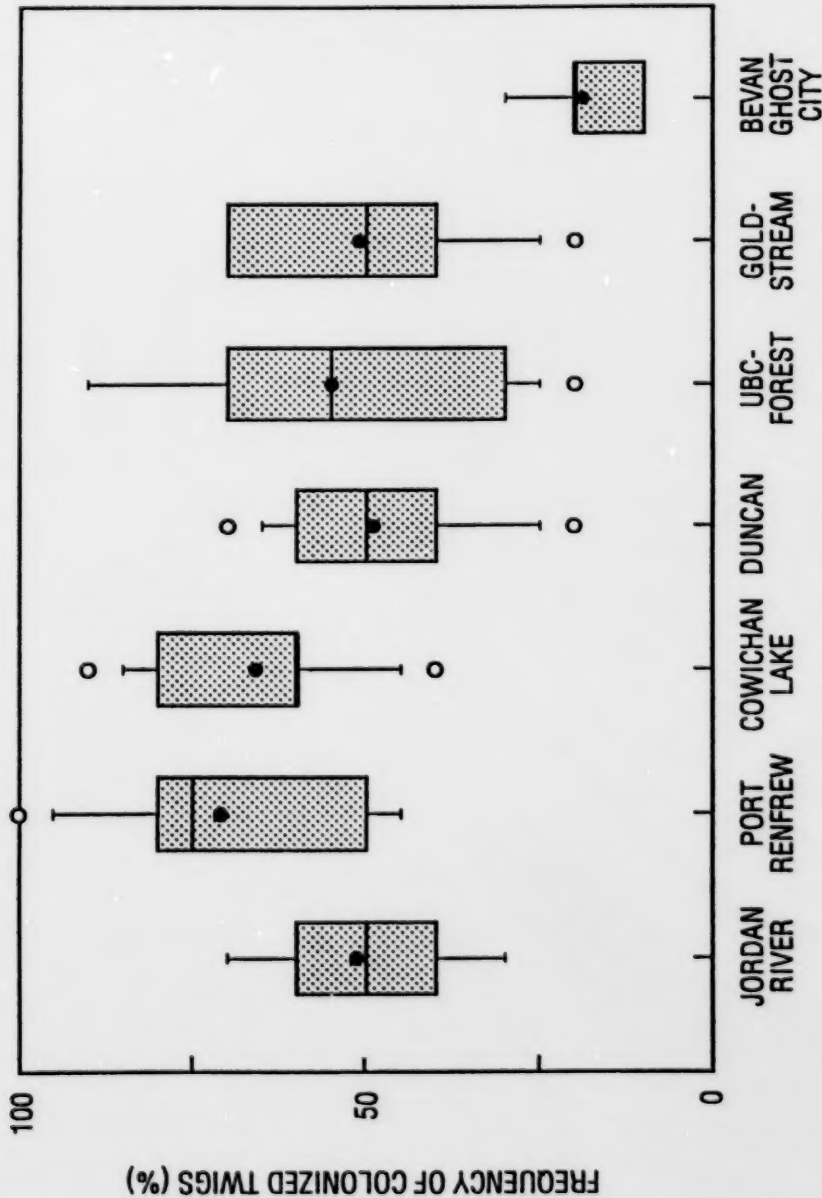


Fig. 2: Box graph displaying the among-tree variation of the colonization of bigleaf maple twigs by endophytic fungi at each site. The horizontal line in the interior of the box indicates the median value. The upper and the lower horizontal lines of the box represent the 75th and the 25th percentiles of the frequency of colonized twigs. If the median value falls exactly on either quartile demarcation, the demarcation line is extra bold. The ends of the vertical bars represent the 10th and the 90th percentiles. Points below or above vertical bars represent values of trees outside the 80% confidence interval. o - mean frequency.

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Cryptodiaporthe hystrix (Anamorph: Diplodina acerina):

Morphology. -Submersed mycelium hyaline, sometimes with black patches; aerial mycelium rare, white; conidiomata stromatic, multilocular, first white becoming darker when older; conidiophores hyaline, septate and branched measuring 18.0-25.0 x 2.5-3.0 μm ; conidiogenous cells hyaline, enteroblastic, phialidic, flask-shaped to cylindrical, often with very distinct collarettes, 6.0-7.0 x 2.5-3.0 μm ; conidia hyaline, fusiform, mostly 1 septate measuring 11.5-17.0 x 2.5-4.0 μm , sometimes without septum and measuring 9.0-14.5 x 2.2-4.0 μm .

The morphology in culture corresponds quite well with that on dead host tissue described by Sutton (1980), except for the greater variation in conidia size in culture. In respect to culture characteristics, however, the strains isolated during this study were quite different from the ones isolated during a preliminary study of the endophyte population of Acer pseudoplatanus in Switzerland (Sieber, unpublished). The Swiss strains always had a lot of green-gray aerial mycelium, and they were isolated mainly from the leaves (up to 95 % of the leaves colonized). Thus, the Swiss and B. C. strains seem to be at least geographical varieties.

Pathogenicity. -Target organs: leaves and twigs. According to Sinclair et al. (1987) C. hystrix is associated with leaf spots and blight of leaves as well as with cankers and diebacks of twigs and branches of several hardwoods, especially of maples. Funk (1981) mentions only the rarely observed C. salicella (Fr.) Petr. (and C.

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salicina [Curr.] Wehm.) which can cause branch cankers of Alnus, Acer, Populus and Salix species. The conidia of C. salicella, however, measure 16.0-20.5 x 4-4.5 μm , i. e. they are larger than the ones of C. hystrix and can therefore not be confused with them. Thus, the species isolated as an endophyte was definitely not C. salicella.

Tests of pathogenicity of C. hystrix conducted at PFC led to contradictory results. None of the C. hystrix strains tested inhibited growth of cambial callus of Acer macrophyllum significantly (Sieber et al. 1990). On the other hand, stem wound infections with C. hystrix strains (all originally isolated as endophytes) induced the formation of circumferential cankers on 60 to 80 % of inoculated 6-month-old A. macrophyllum seedlings (Sieber, unpublished).

Distribution and frequency as endophyte. -Whereas C. hystrix occurred only sporadically in the leaves, it was the main colonizer of twigs at most sites (Table 3). Exceptions were Jordan River and Bevan Ghost City where Phomopsis sp. 1, Glomerella cingulata respectively, dominated. The average frequency of colonization of twigs by C. hystrix was similar at most sites and lay between 25 and 40 % except at Jordan River and Bevan Ghost City. Colonization by C. hystrix at the latter site was significantly lower than at each other site whereas the one at the former was only significantly different from that at Port Renfrew, Duncan, and the UBC-Forest (Mann Whitney U test, $p < 0.05$).

The among-tree variation of the colonization of the twigs

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depended very much on the site and lay between 10 % at Bevan Ghost City and 90 % at Port Renfrew and UBC-Forest (Fig. 3). Except at Duncan, there was at least one tree without C. hystrix at each site (Table 4).

A significant correlation did not exist between colonization by C. hystrix and weather conditions (Table 1).

Table 3: Average frequency (%) of twigs (t) and leaves (l) of bigleaf maple colonized by pathogenic (bold) and frequently isolated non-pathogenic species at each site. 1=Jordan River, 2=Port Renfrew, 3=Cowichan Lake, 4=Duncan, 5=UBC-Forest, 6=Gold Stream, 7= Bevan Ghost City.

Site	Crypto-diaporthe hystrix		Diaporthe eres		Glomerella cingulata		Pezicula livida		Phomopsis sp. 1		Phomopsis sp. 2	
	l	t	l	t	l	t	l	t	l	t	l	t
1 ⁶	4	14	16	6	1	0	0	1	50	16	31	3
2 ⁷	1	38	15	0	2	0	2	21	25	7	8	1
3 ⁷	0	35	46	13	3	0	0	5	16	3	18	6
4	-	38	-	0	-	1	-	1	-	2	-	0
5	-	40	-	0	-	2	-	1	-	12	-	0
6	-	25	-	2	-	4	-	6	-	0	-	2
7	-	4	-	1	-	5	-	0	-	0	-	3

6. only 7 trees (70 leaves and 70 twigs) examined

7. from 10 trees only a total of 30 leaves examined

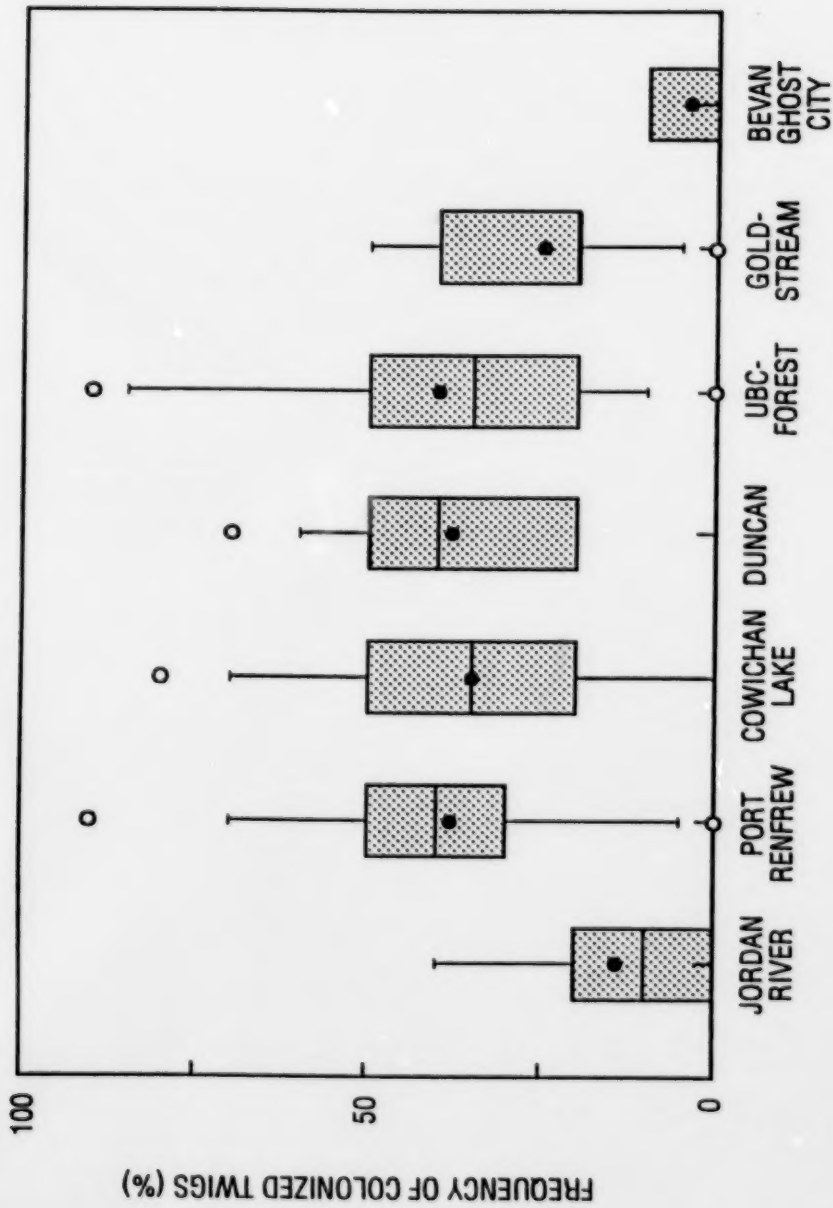


Fig. 3: Box graph displaying the among-tree variation of the colonization of bigleaf maple twigs by *Cryptodiarthe hystrix* at each site. Explanations see Fig. 2.

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Table 4: Frequency (%) of bigleaf maples with at least one leaf (l), twig (t) respectively, colonized by pathogenic (bold) and frequently isolated non-pathogenic species at each site.
Legend: see Table 3.

Site	Crypto-diaporthe hystrix		Diaporthe eres		Glomerella cingulata		Pezicula livida		Phomopsis sp. 1		Phomopsis sp. 2	
	l	t	l	t	l	t	l	t	l	t	l	t
1	40	70	60	30	10	0	0	10	70	90	30	10
2	10	90	60	0	20	0	20	80	70	20	60	10
3	0	80	80	70	20	0	0	20	40	20	60	20
4	-	100	-	0	-	10	-	10	-	20	-	0
5	-	90	-	0	-	10	-	10	-	60	-	0
6	-	90	-	20	-	10	-	40	-	0	-	10
7	-	40	-	10	-	40	-	0	-	0	-	20

Diaporthe eres (Anamorph: Phomopsis occulta):

Morphology. -cultures fast growing, white; aerial mycelium not always present, white; conidiomata stromatic, measuring up to 700 µm in diameter, ostiolate, mostly unilocular, lenticular, inner wall (60-100 µm) consisting of bright to dark brown textura angularis, outer wall consisting of brownish textura intricata; conidiophores hyaline, septate and branched, up to 40.0 x 1.5-2.5 µm; conidiogenous cells hyaline, enteroblastic, phialidic, cylindrical, collarettes distinct, 14.0-20.0 x 1.5-2.0 µm; conidia of two basic types, but often with intermediates between the two: α-conidia hyaline, fusiform, straight, aseptate, 6.5-9.0 x 2.2-3.0 µm and β-conidia hyaline, filiform, slightly curved or straight and very often bent at one end like a walking-stick, 23.0-28.0 x 1.0 µm; hymenium of the upper wall ('roof') of the conidiomata bearing often only α-conidia and hymenium of the lower wall ('floor') bearing β-conidia.

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Phomopsis species are in general very difficult to identify especially when identification is based on the morphology in culture only. The cultural morphology of the species described above, however, did not deviate from that of a species very often isolated as an endophyte from different conifers in Switzerland (Sieber-Canavesi & Sieber 1987, Sieber 1988). This Swiss Phomopsis endophyte of conifers has been identified as P. occulta according to Hahn (1930). During the Swiss studies some of the cultures of P. occulta also formed the teleomorph which has been identified as D. eres.

Pathogenicity. -Target organs: twigs. It occurs around the world in association with cankers and diebacks of hundreds of plant species (Funk 1981, Sinclair et al. 1987). Isolation from cankers and reinoculation of healthy plants, however, did not produce disease symptoms (Sinclair et al. 1987). According to Funk (1981), it is chiefly saprobic and can invade living tissues only from dead plant parts. Thus, its pathogenicity is probably rather low.

Distribution and frequency as endophyte. -Besides Phomopsis sp. 1 and sp. 2 it was one of the main colonizers of the leaves. Except at Cowichan Lake - where it was the second most frequent endophyte of twigs - it occurred only rarely or not at all in the twigs (Table 3). At Cowichan Lake, the colonization of leaves was not significantly higher than at the two other sites because of great between-tree-variations whereas that of twigs was significantly higher than at each other site except at Jordan River.

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No correlations were observed between the frequency of D. eres and weather conditions.

Glomerella cingulata (Anamorph: Colletotrichum gloeosporioides):

Morphology. -cultures very fast growing, gray, sometimes with black patches; aerial mycelium if present gray, floccose; ascomata are ostiolate perithecia and measure 150-400 μm in diameter; asci are clavate-cylindrical, 50.0-60.0 x 6.0-9.0 μm and contain 8 hyaline, ellipsoidal, one-celled ascospores which measure 13.5-17.0 x 4.0-5.5 μm ; surprisingly the Colletotrichum state was observed only once (one conidioma!); conidioma acervular, only one seta observed; conidiophores hyaline, branched only at the base; conidiogenous cells hyaline, enteroblastic, phialidic, cylindrical, 15.0-23.0 x 2.5-4.0 μm ; conidia hyaline, straight, seldom 1-septate, 12.0-21.0 x 4.0-6.0 μm .

Pathogenicity. -Target organs: leaves and twigs. It is one of the most common pathogenic fungi of many different plant species in tropical and temperate regions around the world with the ability to infect at an early stage of plant development and then remain quiescent near the point of penetration for several weeks or months until the plant organ becomes more susceptible (Sinclair et al. 1987). This highly variable species consists of many different varieties that differ in physiological characteristics or host specificity but cannot be distinguished reliably by differences in

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form. Some of the varieties are already used as mycoherbicides (Templeton 1982, TeBeest and Tempelton 1985). Thus, the G. cingulata strains isolated during this study should be involved in the screening process of candidate mycoherbicides, too. It has to be pointed out, however, that G. cingulata is known to cause leaf blight and terminal crook of seedlings of western hemlock (Tsuga heterophylla [Raf.] Sarg.) which is one of the crops to be protected.

Distribution and frequency as endophyte. -This species was only a minor component of the endophyte population of bigleaf maple (Table 2 and 3). It seems not to be very organ specific because it colonized twigs and leaves to the same extent. In this respect it would be the ideal mycoherbicide because it would affect all above-ground parts of bigleaf maple.

Pezicula livida (Anamorph: Cryptosporiopsis abietina):

Morphology. -Cultures rather slow growing, dark green to black, bumpy; aerial mycelium short, occurs mainly on bumps; conidiomata acervular; conidiophores consist of conidiogenous cells only; conidiogenous cells hyaline, entero- or holoblastic, cylindrical, 10.0-14.0 x 3.5-4.5 μm ; conidia hyaline, cylindrical to ellipsoid, straight, 26.0-30.0 x 9.0-10.0 μm ; microconidia only rarely observed.

Cultures with the same characteristics were also obtained from

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red alder (see below).

At least four Pezicula and one Dermea (Dermatea) species on Acer spp. as well as two Pezicula species on Alnus spp. are described to have a Cryptosporiopsis-like anamorphic state: Pezicula acericola (Peck) Sacc., P. carnea (Cooke & Ellis) Rehm, P. spiculata Seaver & Velazquez, P. subcarnea Groves and Dermea acerina (Peck) Rehm on Acer spp., as well as P. aurantiaca Rehm and P. alni Rehm on Alnus spp. (Anonymous 1960, Groves 1938, 1940, and 1941, Seaver and Velazquez 1933). In respect to conidia size, only P. acericola, P. carnea, and P. subcarnea correspond with the species described above. But P. acericola and P. carnea - which are identical in culture and can be separated only by the morphology of the teleomorph - do not fit either, because they are characterized by a fast growing white and fluffy mycelia (Groves 1938 and 1941). P. subcarnea has yellow conidia and can therefore also be excluded.

According to their cultural and morphological characteristics, the isolates found on bigleaf maple and red alder resemble those from conifers and hardwoods in Switzerland which have been identified as Cryptosporiopsis abietina (Sieber and Hugentobler 1987, Sieber-Canavesi and Sieber 1987, Sieber 1989). Some of the strains isolated from Fagus sylvatica formed even the teleomorph in culture. This conformed with Pezicula livida.

Pathogenicity. -Target organs: twigs. To our knowledge, it has never been reported to cause diseases. Funk (1981) mentions it to be a secondary invader of cankers and diebacks of Douglas fir and western hemlock. According to Carroll (1988) Cryptosporiopsis spp.

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are among the genera of fungi which always appear in endophyte censuses regardless of the host plant sampled. Thus, P. livida has to be considered rather to be a neutral if not beneficial symbiont than a parasite.

Distribution and frequency as endophyte. -It is rather a colonizer of twigs than of leaves (Table 2 and 3).

It occurred most frequently at Port Renfrew where the amount and frequency of rain was highest (Table 1). The high percentage of twigs colonized by P. livida at Port Renfrew could therefore be due to the abundance of rain. This site is also characterized by two other extremes: the mean summer temperature and the number of days of frost! These two factors could have had an influence on the colonization by this fungus as well.

It has to be pointed out, however, that P. livida did not occur at all in red alder at Port Renfrew!

Phomopsis sp. 1:

Morphology. -Colony first colorless (sometimes with a touch of pink) with some very small radial brown strips, aerial mycelium absent; older cultures darkbrown to black with thin layers of gray floccose aerial mycelium; stromata multilocular and of two types - type 1: tree-like, up to 5 mm long, stem 1.5 mm wide consisting of *textura angularis* and *intricata*, branched, conidiogenous loci at the end of the branches, loci usually not sphaerical and 30-200 μ m in

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diameter - type 2: stroma not tree-like, up to 400 μm high and 1 mm wide consisting of darkbrown, thick-walled textura angularis, conidiogenous loci close to the surface of the stroma, usually sphaerical and measuring 60-120 μm in diameter; conidiophores hyaline, septate and branched, at least 30 μm long; conidiogenous cells hyaline, enteroblastic, phialidic, cylindrical; conidia of two basic types: γ -conidia hyaline, ellipsoid to fusiform, straight, aseptate, 6.0-9.0 x 2.0-2.5 μm and β -conidia hyaline, filiform, straight or falcate, 13.0-21.0 x 1.0-1.2 μm (tree-like typus) or 22.0-29.0 x 1.0 μm (flat typus).

This species occurred as an endophyte on red alder, too (see below).

Uecker (1988) lists 12 different Phomopsis species which are reported to occur on either Alnus or Acer spp.. None of these species, however, fits the above described species. It has been decided, therefore, to assign a new species name to this fungus.

Pathogenicity. -Target organs: leaves and twigs. As long as infection experiments do not prove otherwise, Phomopsis sp. 1 has to be considered non-pathogenic.

Distribution and frequency as endophyte. -It was the most frequent endophyte of the leaves at Jordan River and Port Renfrew. At Jordan River, it was also the most frequently isolated endophyte of twigs (Table 3).

Correlations between the frequency of colonization by Phomopsis sp. 1 and any of the weather components could not be observed.

Phomopsis sp. 2:

Morphology. -Colonies in the centre black becoming gray towards the margin, spotted with white crystals (most conspicuous!); aerial mycelium rare if present; basal stroma of dark thick-walled textura angularis, approx. 500 μm in diameter; conidioma bearing stroma of brownish textura intricata, approx. 200 μm wide and 180 μm high; conidiomata single, sphaerical, surrounded by a thin layer of textura oblita, up to 140 μm in diameter; conidiophores hyaline, septate and branched, up to 40.0 x 1.0-2.5 μm ; conidiogenous cells hyaline, enteroblastic, phialidic, cylindrical, collarettes distinct, up to 20.0 x 1.0-2.0 μm ; conidia of two types: α -conidia, hyaline, ellipsoid to fusiform, straight, aseptate, rare, 6.5-9.0 x 2.0-3.0 μm and β -conidia, hyaline, filiform, straight or (rarely!) slightly falcate, 11.0-15.0 x 1.0 μm .

This species has been isolated very often from red alder, too!

In respect to the species name of this fungus the statement already given for Phomopsis sp. 1 applies, too.

Pathogenicity. -See Phomopsis sp. 1!

Distribution and frequency as endophyte. -Besides D. eres (Phomopsis occulta) and Phomopsis sp. 1, it is one of the main components of the endophyte population of leaves of bigleaf maple (Table 3 and 4).

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None of the weather factors considered influenced the frequency of this fungus significantly (Table 1).

Latent Disease Potential (LDP) for the seven bigleaf maple stands

LDPs of leaves were rather low in respect to the ones of the twigs (Fig. 4). LDP of twigs depended mainly on the frequency of colonization by C. hystrix.

Of course, the LDPs calculated herein do not reflect the real degree of latent diseases adequately, yet. More information has to be sampled and involved in the LDP-equation. First of all, the relative pathogenicity of each species has to be evaluated by infection experiments. Secondly, twig segments belonging to different age-classes differ in the frequency of colonization by endophytes (Petrini and Müller 1979, Sieber-Canavesi and Sieber 1987): in addition to 2- to 3-yr-old twig segments younger as well as older segments should therefore be investigated as well.

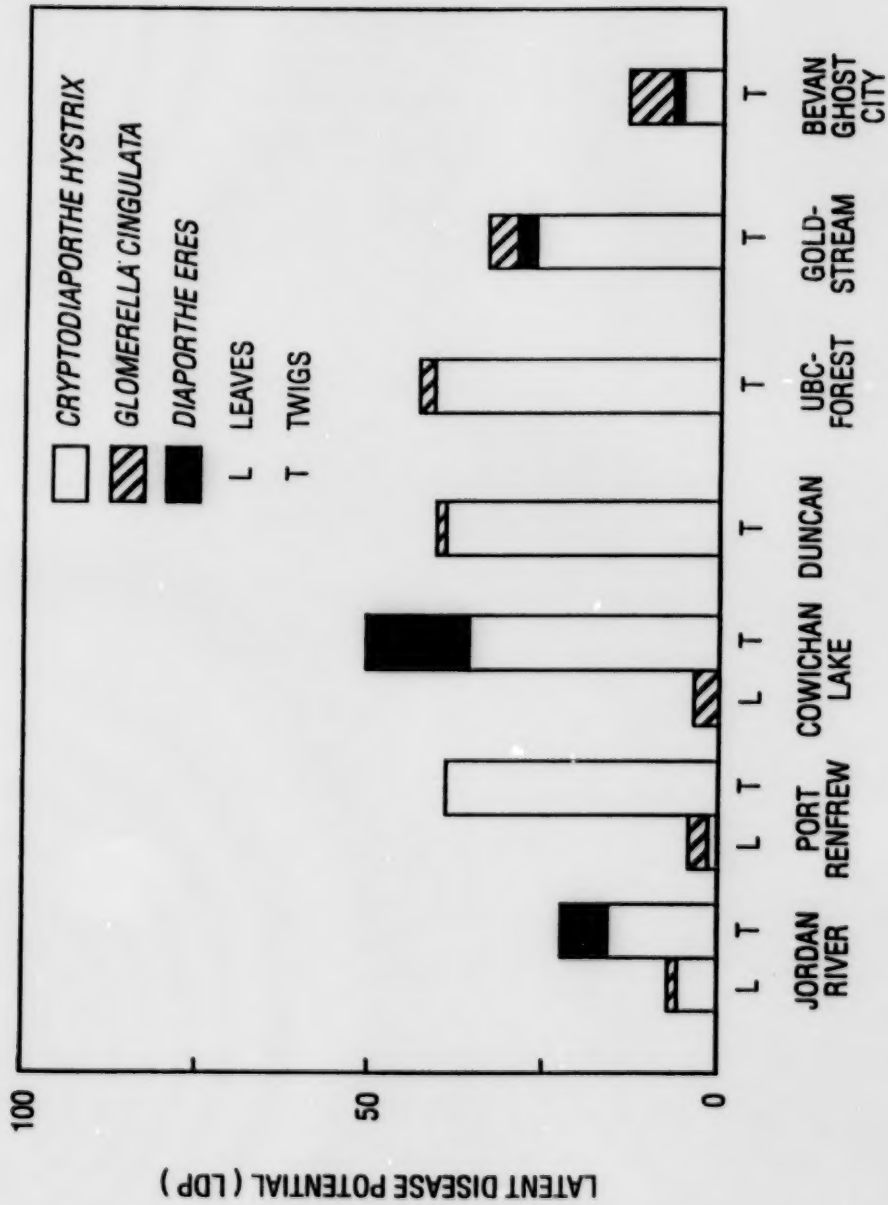


Fig. 4: Latent disease potential of bigleaf maple at each site.

RED ALDER

Overall colonization

Ninety percent of all leaves and 83 % of all twigs were colonized by at least one endophytic fungus (Table 5). More than one third of the colonized leaves and almost one fifth of the colonized twigs bore two or more species.

Table 5: Frequency of colonization of leaves and twigs of red alder by endophytic fungi.

<u>Taxon</u>	i.c. ⁸	frequency (%) of colonization of leaves twigs	
Number of leaves/twigs examined		300	800
Percentage of leaves/twigs colonized by endophytic fungi		90.0	83.0
-colonized by one species		56.7	64.3
-colonized by two species		30.3	17.5
-colonized by three species		3.0	1.1
-colonized by four species		0	0.1
<u>Ascomycetes</u>			
<u>Coniochaeta</u> sp., anamorph: <u>Phialophora</u>	a+t ⁹	0.7	0.3
<u>Diaporthe</u> <u>eres</u> Nitschke, anam.:			
<u>Phomopsis</u> <u>occulta</u> (Sacc.) Trav.	a	7.0	2.4
<u>Diaporthe</u> sp.	t	0	0.1
<u>Gnomonia</u> <u>setacea</u> (Pers.: Fr.) Ces. & de Not.	t	39.7	0.5
<u>Gnomoniella</u> <u>tubaeformis</u> (Fr.) Sacc., anam.:			
<u>Asteroma</u> <u>alneum</u> (Pers.: Fr.) Sutton	a	22.3	5.1
<u>Hypoxylon</u> <u>bipapillatum</u> Berk., anam.: <u>Geniculosporium</u>	s	0.7	0
<u>Hypoxylon</u> <u>irregulare</u> Cke., anam.: <u>Geniculosporium</u>	a	0.3	0
<u>Hypoxylon</u> <u>serpens</u> (Pers.: Fr.) Kickx, anam.: <u>Geniculosporium</u>	a	0.3	0.6

8. a = anamorph formed, t = teleomorph formed, s = sterile

9. only sterile ascomata formed

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Table 5 continued

<u>Taxon</u>	i.c.	frequency (%) of	
		colonization of	leaves twigs
<u>Melanconis alni</u> Tul., anam.: <u>Melanconium sphaeroideum</u> Link: Fr.	a		2.0
<u>Mycosphaerella punctiformis</u> (Pers.: Fr.) Schroet., anam.: <u>Ramularia</u>	a	7.7	0
<u>Nectria</u> sp.	t	0	0.8
<u>Ophiovalsa suffusa</u> (Fr.) Petrak, anam.: <u>Disculina vulgaris</u> (Fr.) Sutton	a	1.3	13.0
<u>Pezicula carpinea</u> (Pers.: Pers.) Tul.: Fckl., anam.: <u>Cryptosporiopsis</u> <u>fasciculata</u> (Tode: Tul.) Petrak	a	0	0.1
<u>Pezicula livida</u> (Berk. & Br.) Rehm, anam.: <u>Cryptosporiopsis abietina</u> Petrak	a	2.0	6.3
<u>Pleospora herbarum</u> (Pers.: Fr.) Rabenh.	t	0.3	0
<u>Xylaria</u> sp.	s	1.7	3.4
Deuteromycetes			
Coelomycetes			
<u>Asteromella</u> sp.		0	0.1
<u>Coniothyrium</u> sp.		0.3	0
<u>Cryptocline</u> sp.		0	0.1
<u>Cryptosporiopsis</u> sp.		0.7	4.1
<u>Libertella</u> sp.		0	0.1
<u>Melanconium</u> sp.		0	0.3
<u>Phoma</u> spp.		2.0	1.0
<u>Phomopsis</u> sp. 1		3.3	1.9
<u>Phomopsis</u> sp. 2		11.0	16.5
<u>Phomopsis</u> sp. 3		1.0	0
<u>Septoria alni</u> Sacc.		2.3	0
Hyphomycetes			
<u>Aureobasidium pullulans</u> (de Bary) Arn.		1.0	0.5
<u>Botrytis</u> sp.		0	0.3
<u>Cladosporium cladosporioides</u> (Fresen.) de Vries		0.3	0
<u>Cladosporium sphaerospermum</u> Penz.		2.7	2.6
<u>Nodulisporium</u> sp. 1		0.7	0
<u>Nodulisporium</u> sp. 2		1.3	0.1
<u>Oedocephalum</u> sp.		0	0.1
<u>Periconiella</u> sp.		0.3	0.1
<u>Phialophora hoffmannii</u> (Beyma) Scholl-Schwarz		0.3	0.6
<u>Rhizoctonia</u> sp.		0	0.6
<u>Trichoderma</u> sp.		0.3	0
Sterile Mycelia			
Black Mycelium 1		0	2.1
Black Mycelium 2		0	8.8

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Table 5 continued

<u>Taxon</u>	frequency (%) of	
	colonization of leaves	twigs
Brown Mycelium	0	6.3
Yellow Mycelium	0	4.6
Others	14.7	17.8

Depending on the site, overall colonization varied between 82 (Jordan River) and 97 % (Port Renfrew) for leaves as well as between 77 (Jordan River) and 95 % (Cowichan Lake) for twigs. Overall colonization of leaves by endophytic fungi at Jordan River was significantly lower than that at the two other sites (Mann-Whitney U, $p < 0.05$). The colonization of twigs at Cowichan Lake was significantly higher than that at other sites with the exception of Duncan and Gold Stream (Fig. 5). On the other hand, Port Renfrew showed an overall frequency of colonized twigs which was significantly lower than that at Cowichan Lake, Duncan and Gold Stream.

The among-tree variation was highest at UBC-Forest, Bevan Ghost City and Campell River (50-100 %) and lowest at Cowichan Lake (90-100 %) (Fig. 5).

The overall colonization of twigs and the amount of snow correlated significantly and positively (Fig. 6). Overall colonization increased by 8.5 % per 100 mm snowfall.

Similarity of mycoflora within twigs and leaves

According to Table 5, the similarity index λ is 0.62 (only species with frequency ≥ 0.3 % included in calculation) indicating that the endophyte populations of twigs and leaves of red alder displayed

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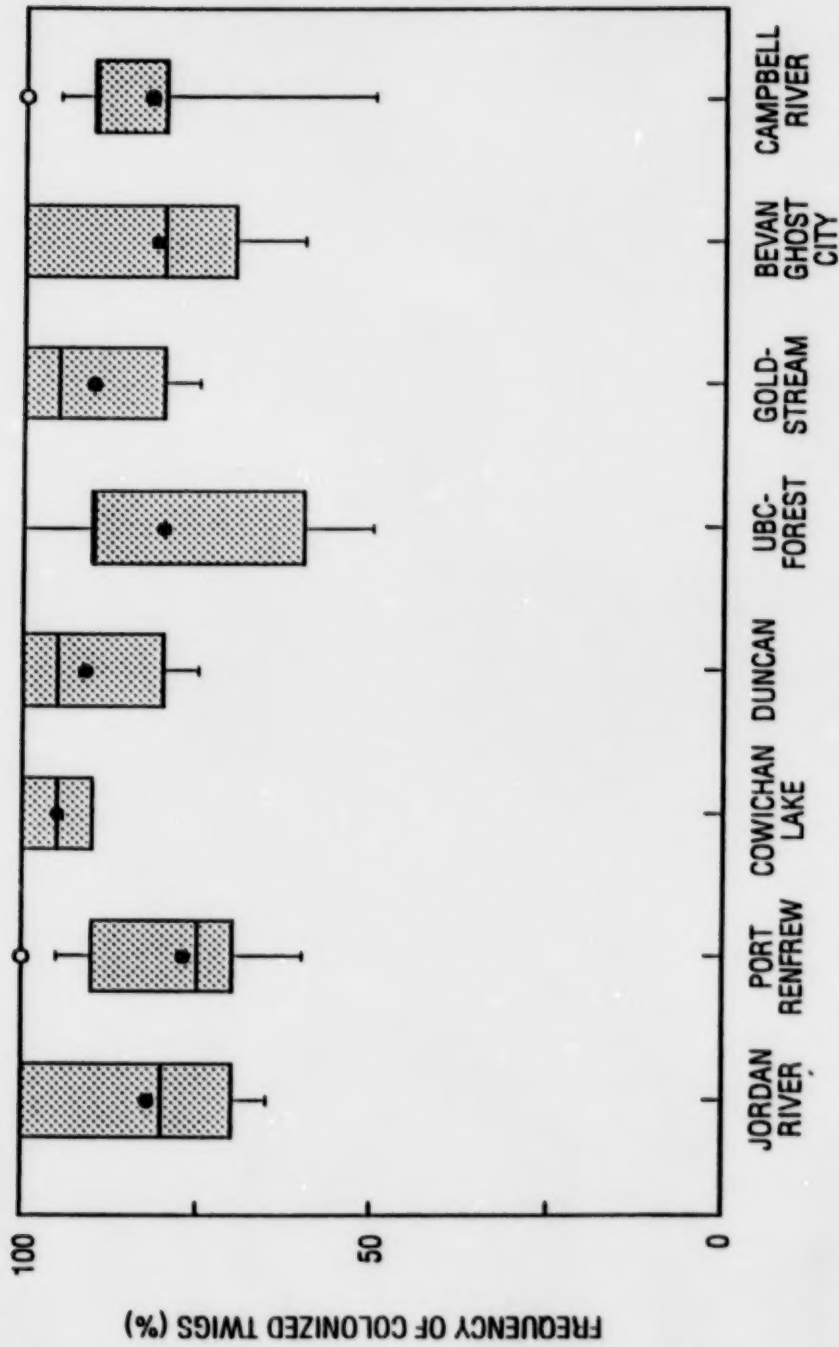


Fig. 5: Box graph displaying the between-tree variation of the colonization of red alder twigs by endophytic fungi at each site. Explanations see Fig. 2.

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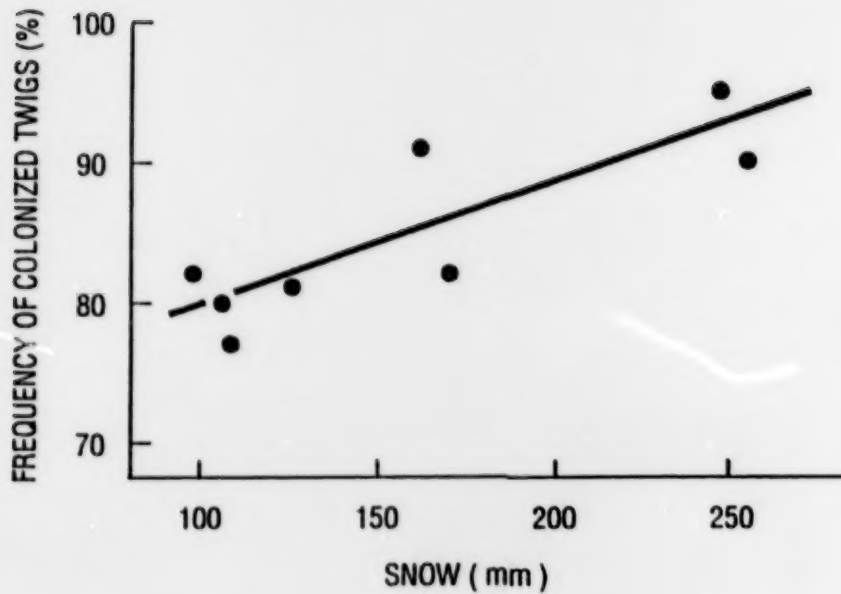


Fig. 6: Effect of snow on the overall colonization of red alder twigs by endophytic fungi: Overall colonization [%] = $0.0853 \cdot \text{snow [mm]} + 71.1141$ ($s_{\text{slope}} = 0.0226$, $s_{\text{intercept}} = 3.8380$).

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greater variation than the ones of bigleaf maple. Only a few more than half the number of species occurring on the leaves and twigs, respectively, occurred also on the other organ.

Colonization by frequently isolated and known pathogenic species

The endophyte population of the leaves was dominated by Gnomonia setacea, Gnomoniella tubaeformis, and Phomopsis sp. 2 (Table 5). These species occurred in more than 10 % of the leaves. Except Diaporthe eres and Mycosphaerella punctiformis, all other species were present in less than 5 % of the leaves. Ophiovalsa suffusa and Phomopsis sp. 2 were the only two species which occurred in more than 10 % of the twigs. Gnomoniella tubaeformis, Pezicula livida, the Black Mycelium 1, and the Brown Mycelium colonized more than 5 % of the twigs.

Six potentially pathogenic species could be isolated from either one or both of the leaves and twigs: D. eres, G. setacea, G. tubaeformis, Melanconis alni, Nectria sp., and Septoria alni.

The species mentioned above are described and discussed below in respect to their morphology, taxonomy, pathogenicity, distribution and frequency as endophytes:

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Diaporthe eres (Anamorph: Phomopsis occulta):

Morphology and Pathogenicity see chapter 'BIGLEAF MAPLE'

Distribution and frequency as endophyte. -It was a quite frequent colonizer of both leaves and twigs (Table 5 and 6). The sites were not significantly different in respect to its frequency.

Table 6: Average frequency (%) of twigs (t) and leaves (l) of red alder colonized by endophytic fungi known to be pathogenic at each site. 1=Jordan River, 2=Port Renfrew, 3=Cowichan Lake, 4=Duncan, 5=UBC-Forest, 6=Gold Stream, 7=Bevan Ghost City, 8=Campell River.

Site	Diaporthe eres		Gnomonia setacea		Gnomoniella tubaeformis		Melanconis alni		Nectria sp.		Septoria alni	
	l	t	l	t	l	t	l	t	l	t	l	t
1	9	1	34	0	2	3	0	1	0	0	0	0
2	5	2	43	1	51	8	0	0	0	0	6	0
3	7	7	42	0	14	13	0	5	0	1	1	0
4	-	3	-	1	-	0	-	0	-	2	-	0
5	-	4	-	0	-	2	-	0	-	2	-	0
6	-	1	-	0	-	9	-	7	-	0	-	0
7	-	1	-	2	-	4	-	0	-	0	-	0
8	-	0	-	0	-	2	-	3	-	1	-	0

Table 7: Frequency (%) of twigs (t) and leaves (l) of red alder colonized by frequently isolated non-pathogenic endophytes.

Site	Mycosphaerella punctiformis		Ophiovalsa suffusa		Pezicula livida		Phomopsis sp. 2	
	l	t	l	t	l	t	l	t
1	2	0	2	18	1	16	9	5
2	6	0	0	3	0	0	1	9
3	15	0	2	6	5	8	23	41
4	-	0	-	10	-	7	-	8
5	-	0	-	13	-	1	-	0
6	-	0	-	23	-	4	-	12
7	-	0	-	8	-	1	-	43
8	-	0	-	23	-	13	-	13

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Table 8: Frequency (%) of red alders with at least one leaf (l), twig (t) respectively, colonized by endophytic species known to be pathogenic.

Site	Diaporthe eres		Gnomonia setacea		Gnomoniella tubaeformis		Melanconis alni		Nectria sp.		Septoria alni	
	l	t	l	t	l	t	l	t	l	t	l	t
1	40	10	100	0	20	20	0	10	0	0	0	0
2	40	20	100	10	90	60	0	0	0	0	40	0
3	40	20	100	0	60	70	0	40	0	10	10	0
4	-	20	-	10	-	0	-	0	-	20	-	0
5	-	40	-	0	-	20	-	0	-	20	-	0
6	-	10	-	0	-	80	-	60	-	0	-	0
7	-	10	-	20	-	20	-	0	-	0	-	0
8	-	0	-	0	-	20	-	20	-	10	-	0

Table 9: Frequency (%) of red alders with at least one leaf (l), twig (t) respectively, colonized by frequently isolated non-pathogenic endophytes.

Site	Mycosphaerella punctiformis		Ophiovalsa suffusa		Pezicula livida		Phomopsis sp. 2	
	l	t	l	t	l	t	l	t
1	20	0	20	90	10	50	40	30
2	50	0	0	30	0	0	10	60
3	70	0	20	40	20	50	90	100
4	-	0	-	50	-	40	-	50
5	-	0	-	70	-	10	-	0
6	-	0	-	100	-	40	-	70
7	-	0	-	50	-	10	-	90
8	-	0	-	100	-	100	-	80

Gnomonia setacea:

Morphology. -submersed mycelium hyaline; aerial mycelium rarely observed, white; perithecia 150-250 µm in diameter with very long ostioli (up to 500 µm); asci 35.0-40.0 x 6.0-7.5 µm containing 8 spores; ascospores hyaline, fusiform 1-septate, 14.2-15.8 x 1.8-2.2 µm.

Pathogenicity. -Target organs: leaves. Funk (1985) describes this

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species to produce leaf browning and early abscission (anthracnose) on Alnus spp.. According to other authors it occurs especially on overwintered leaves (Barr 1978, Monod 1983).

Distribution and frequency as endophyte. -Except at Port Renfrew, G. setacea was the most frequent colonizer of the leaves (Table 6). The sites were not significantly different in respect to red alder colonization by this fungus.

Gnomoniella tubaeformis (Anamorph: Asteroma alneum, formerly described as Gloeosporium sp. 1 [Sieber et al. 1989]):

Morphology. -submersed mycelium hyaline, becoming slightly brown in the centre when older; aerial mycelium lacking; darkbrown conidiomata initials (stroma) all over the colony; conidiomata acervular; conidiophores consisting of the conidiogenous and basal cell; conidiogenous cells hyaline, phialidic, cylindrical to flask-shaped, 10.0-14.0 x 2.0-3.5 μm ; conidia hyaline, mostly one-celled (some 1-septate), variable in shape, cylindrical, fusiform, straight or curved, 9.0-16.0 x 2.2-3.5 μm ; seldom also microconidia present, 6.0-8.0 x 1.5-2.0 μm .

Some descriptions of the anamorphic state of this species do not correspond with each other. Whereas the conidia measure 10.5-13.5 x 3.0 μm (conidiogenous cells: 4.0-6.0 x 3.0-4.0 μm) according to Sutton (1980), their dimensions are 8.0-12.0 x 1.5-2.0 μm (conidiogenous cells: 7.0-10.0 x 2.0-2.5 μm) according to Monod (1983). Either Monod

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and Sutton did not look at the same species or its morphology is really very variable.

Pathogenicity. -Target organs: leaves. In Klebahns (1908) infection experiments, it was shown to be a parasite which infects alder leaves quite easily where it causes leaf spotting and lessions (Barr 1978, Sutton 1980).

Distribution and frequency as endophyte. -The frequency of leaves colonized by G. tubaeformis was significantly different at each site (Mann Whitney U test, $p < 0.05$). It was lowest at Jordan River with 2 % and greatest at Port Renfrew with 51 % (Table 6). Colonization of twigs was highest at Cowichan Lake.

Melanconisalni (Anamorph: Melanconium sphaeroideum):

Morphology. -cultures slow growing, compact, mycelium white, becoming grey-black where sporulation is in progress; aerial mycelium woolly but not very well developed; fast sporulating; conidiomata acervular; conidiophores hyaline, septate, branched, very long, up to $50 \times 1.5-2.0 \mu\text{m}$; conidiogenous cells hyaline, cylindrical, phialidic, sometimes annellidic, $20.0-25.0 \times 1.5-2.0 \mu\text{m}$; conidia hyaline at first, becoming deep dark when older, irregular in shape, ovoid to ellipsoid, $10.0-13.0 \times 5.0-8.0 \mu\text{m}$.

According to Funk (1981) these morphological characteristics correspond rather with the description of Melanconium bicolor Ness

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than with the one of Melanconium sphaeroideum. M. bicolor has long conidiophores and annellidic conidiogenous cells like the species found as endophyte. But it differs from the latter by the presence of a Libertella-state (formed in culture) and by its host; it is only reported from Betula species. The name M. sphaeroideum seemed more appropriate in respect to the host, the lack of a Libertella-state and the great variability of conidiophore length.

Pathogenicity. -Target organs: twigs. M. alni is known to be associated with branch dieback of red alder (Funk 1981). According to Dorworth (1988) it is associated with alder mortality in excess of 90 % through most the range of red alder on the Pacific Coast.

Distribution and frequency as endophyte. -It was only a minor component of the mycoflora of twigs (Table 6).

The percentage of twigs colonized by M. alni and the amount of snow were significantly correlated (Fig. 7): The percentage of twigs colonized increases by 4 % per 100 mm additional snow. It must be pointed out, however, that the position of the regression line is strongly influenced by only two data pairs (Cowichan Lake and Gold Stream). Thus, the importance of this result may not be overestimated.

Mycosphaerella punctiformis (Anamorph: Ramularia):

Morphology. -Colonies slow growing, white; aerial mycelium white, short, powdery; conidiophores hyaline, septate, branched;

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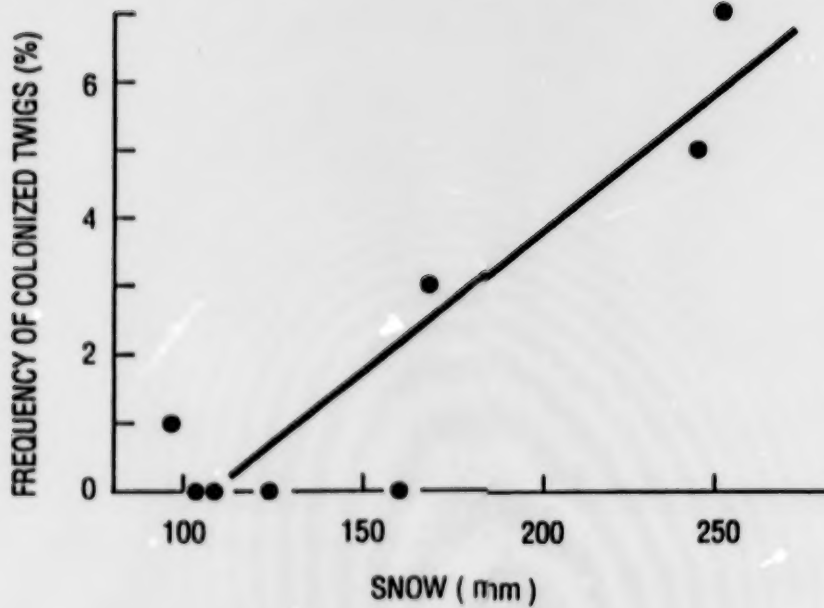


Fig. 7: Effect of snow on the colonization of red alder twigs by *Melanconis alni*: $FM [\%] = 0.0395 \cdot snow [mm] - 4.3069$ ($s_{slope} = 0.0075$, $s_{intercept} = 1.2663$), FM - Frequency of twigs colonized by *M. alni*.

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conidiogenesis holoblastic, forming acropetal chains of conidia; conidia hyaline, cylindrical to ellipsoid, distinct scars at one or both ends (depends on the position of the conidia: if intermediate - two or three, if terminal - only one scar), smooth or slightly verrucose, usually one-celled, sometimes 1-septate or 1-5 pseudoseptate, $6.0-14.0 \times 2.5-4.0 \mu\text{m}$.

Pathogenicity. -Target organs: leaves. According to Müller and von Arx (1962) it is a saprophyte. In spring, its ascospores germinate immediately after they were ejected from the asci and enter into leaf tissues without producing any disease symptoms.

Distribution and frequency as endophyte. -It seems to be very organ specific because it colonized only leaves. At Cowichan Lake it was one of the most frequently isolated endophytes (Table 7 and 9).

Nectria sp.

Morphology. -Cultures very slow growing, tough, slimy, pink to red; aerial mycelium absent; perithecia approx. $500 \mu\text{m}$ in diameter with 1 to 1.2 mm long ostioli, walls hyaline to red; asci unitunicate, $100.0-110.0 \times 16.0-18.0 \mu\text{m}$, containing 8 two-celled ascospores which measure $30.0-34.0 \times 9.5-10.0 \mu\text{m}$; the spores are constricted at the septum, surrounded by gelatinous sheath and bear an appendage at each end; these are $1 \mu\text{m}$ broad and up to $20.0 \mu\text{m}$ long.

This species conforms neither with N. cinnabarina (Tode: Fr.) Fr.

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nor with N. ditissima Tul. which are known to occur quite frequently on Alnus spp. in British Columbia (Funk 1981).

Pathogenicity. -Target organs: twigs. Although most of the known Nectria species have pathogenic properties (diebacks and cankers), the ones of this not yet clearly identified species have first to be evaluated by infection experiments. The pathogenicity of Nectria ditissima Tul. on red alder is currently in test (Dorworth 1988).

Distribution and frequency as endophyte. -It occurred only sporadically in the twigs at four of eight sites (Tables 6 and 8).

Ophiovalsa suffusa (Anamorph: Disculina vulgaris):

Morphology. -Colonies slow growing, submersed mycelium hyaline, sometimes with black spots; aerial mycelium woolly, white to cream colored if present; conidiomata acervular; conidiophores hyaline, septate, branched at the base, 15.0-25.0 x 2.0-4.0 μ m; conidiogenous cells hyaline, annellidic; conidia hyaline, one-celled, falcate to sigmoid, base truncate, 22.0-46.0 x 3.5-6.0 μ m.

O. femoralis (Peck) Petrak is an other species known to occur on dead branches of red alder in the Pacific Northwest (Glawe and Jensen 1986) but since an anamorphic state of this species has not been found, O. suffusa is still the most appropriate name for the species described above (Petrak 1965).

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Pathogenicity. -Target organs: twigs. It is a cosmopolitan species in branches of Alnus sp. and has so far never been reported to cause diseases (Barr 1978).

Distribution and frequency as endophyte. -O. suffusa was the second most frequently isolated endophyte of the twigs (Table 5).

The percentage of twigs colonized by O. suffusa varied very much among trees at most sites (Fig. 8). The within-site variation reached up to 40 %. Average colonization was highest at Gold Stream and Campell River (23 %) and lowest at Port Renfrew (3 %).

Pezicula livida (Anamorph: Cryptosporiopsis abietina):

Morphology and Pathogenicity see chapter 'BIGLEAF MAPLE'

Distribution and frequency as endophyte. -It occurred in both twigs and leaves (Table 5). Whereas it did not occur at all at Port Renfrew, it was one of the main colonizer of twigs at Jordan River and Campell River (Table 7 and 8).

Phomopsis sp. 2:

Morphology and Pathogenicity see chapter 'BIGLEAF MAPLE'

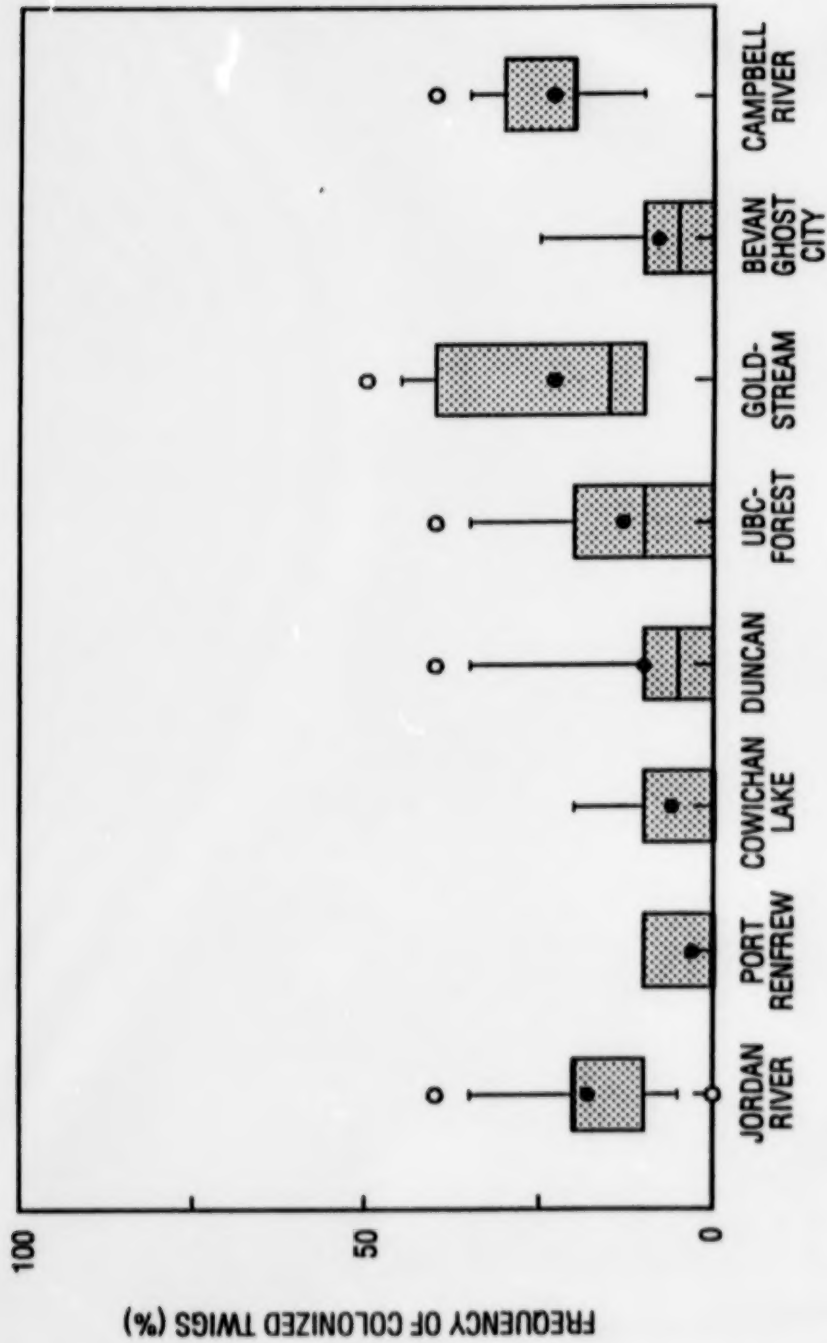


Fig. 8: Box graph displaying the among-tree variation of the colonization of red alder twigs by *Ophiostoma sulfusa* at each site. Explanations see Fig. 2.

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Distribution and frequency as endophyte. -It occurred very frequently in both the leaves and the twigs (Table 5).

The frequency of twigs colonized was significantly higher at Cowichan Lake and at Bevan Ghost City than at any other site ($p < 0.05$) (Fig. 9). Phomopsis sp. 2 did not occur at all in twigs from the UBC-forest. Colonization of twigs at UBC-Forest was significantly different from all other sites but Jordan River ($p < 0.05$). There was no correlation between the frequency of colonization by Phomopsis sp. 2 and any of the meteorological factors.

Septoria alni:

Morphology. -Colonies very slow growing; aerial mycelium short, velvety, green-gray with black spots; surrounding agar red stained; conidiomata pycnidial; conidiogenous cells holoblastic; conidia hyaline, filiform, 3-6-septate, straight or curved, $35.0-47.0 \times 1.8-2.0 \mu\text{m}$.

Pathogenicity. -Target organs: leaves. According to Funk (1985), it causes leaf spot of alder.

Distribution and frequency as endophyte. -It occurred only sporadically in the leaves (Table 6 and 8).

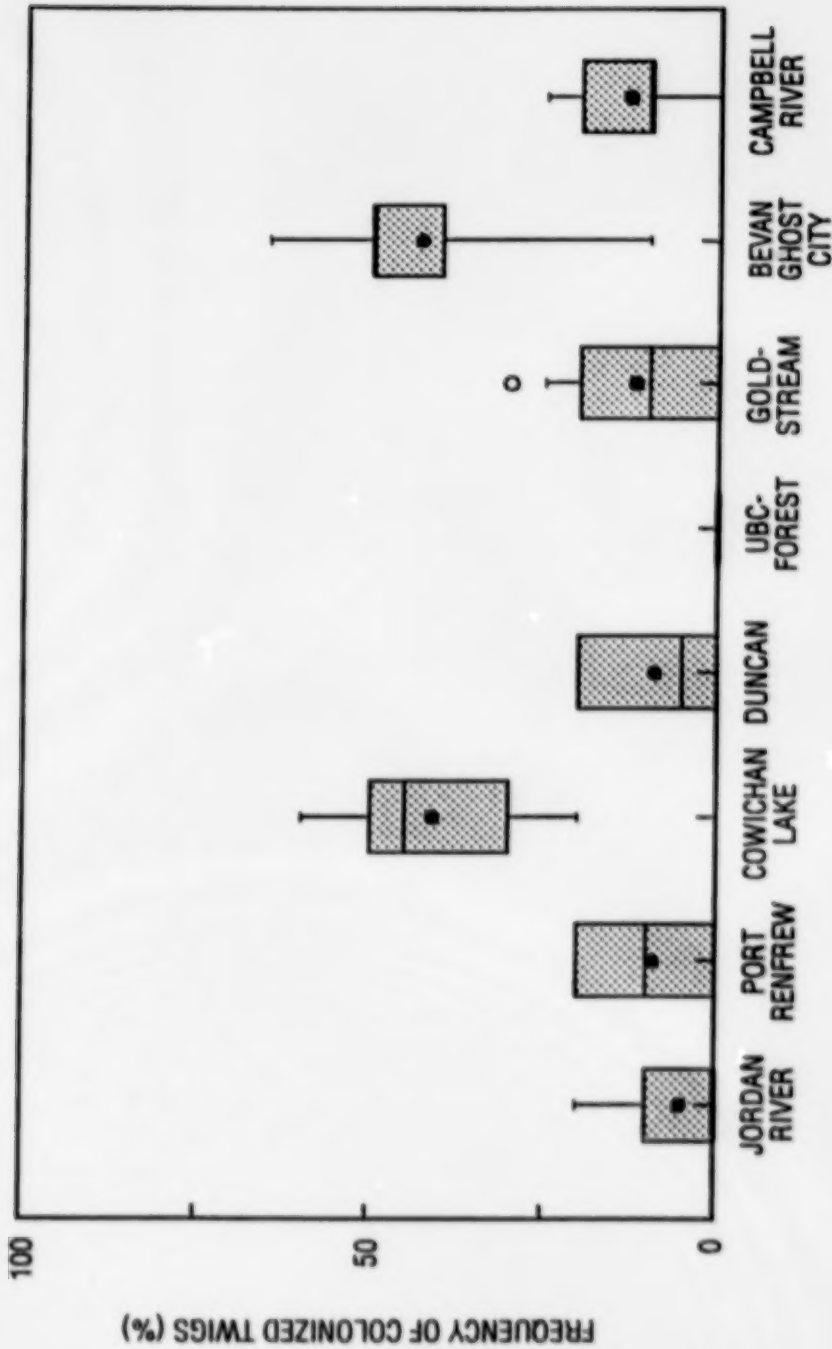


Fig. 9: Box graph displaying the among-tree variation of the colonization of red alder twigs by *Phomopsis* sp. 2 at each site. Explanations see Fig. 2.

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Estimation of LDP at the eight red alder stands

In contrast to bigleaf maple, the LDPs of red alder leaves were always much higher than the LDPs of red alder twigs (Fig. 10). LDPs of the twigs were greatest at Cowichan Lake and Gold Stream (Fig. 10). This was due to the comparatively great colonization by D. eres and M. alni at these sites.

LDP of leaves was highest at Port Renfrew (Fig. 10) because of the great colonization by G. tubaeformis (Fig. 10 and Table 6).

Conclusions

The examination of symptomless bigleaf maple and red alder tissues on the occurrence of endophytic fungi is a useful tool in finding candidate mycoherbicides. Three potentially pathogenic fungi have been found on bigleaf maple: Cryptodiaporthe hystrix, Diaporthe eres, and Glomerella cinquilata; and six on red alder: Diaporthe eres, Gnomonia setacea, Gnomoniella tubaeformis, Melanconis alni, Nectria sp., and Septoria alni. Pathogens expected but not found were Cristulariella depraedans (Cooke) Hoehn., Mycosphaerella mycopappi Funk & Dorworth, Nectria cinnabarina (Tode: Fr.) Fr., and Rhytisma punctatum (Pers.) Fr. on maple as well as Cercospora alni Dearn. & Barth, Didymosphaeria oregonensis Goodding, Encoelia furfuracea (Roth: Pers.) Karst., Melanconis thelebola (Fr.) Sacc., Nectria cinnabarina, and N. ditissima Tul. on red alder.

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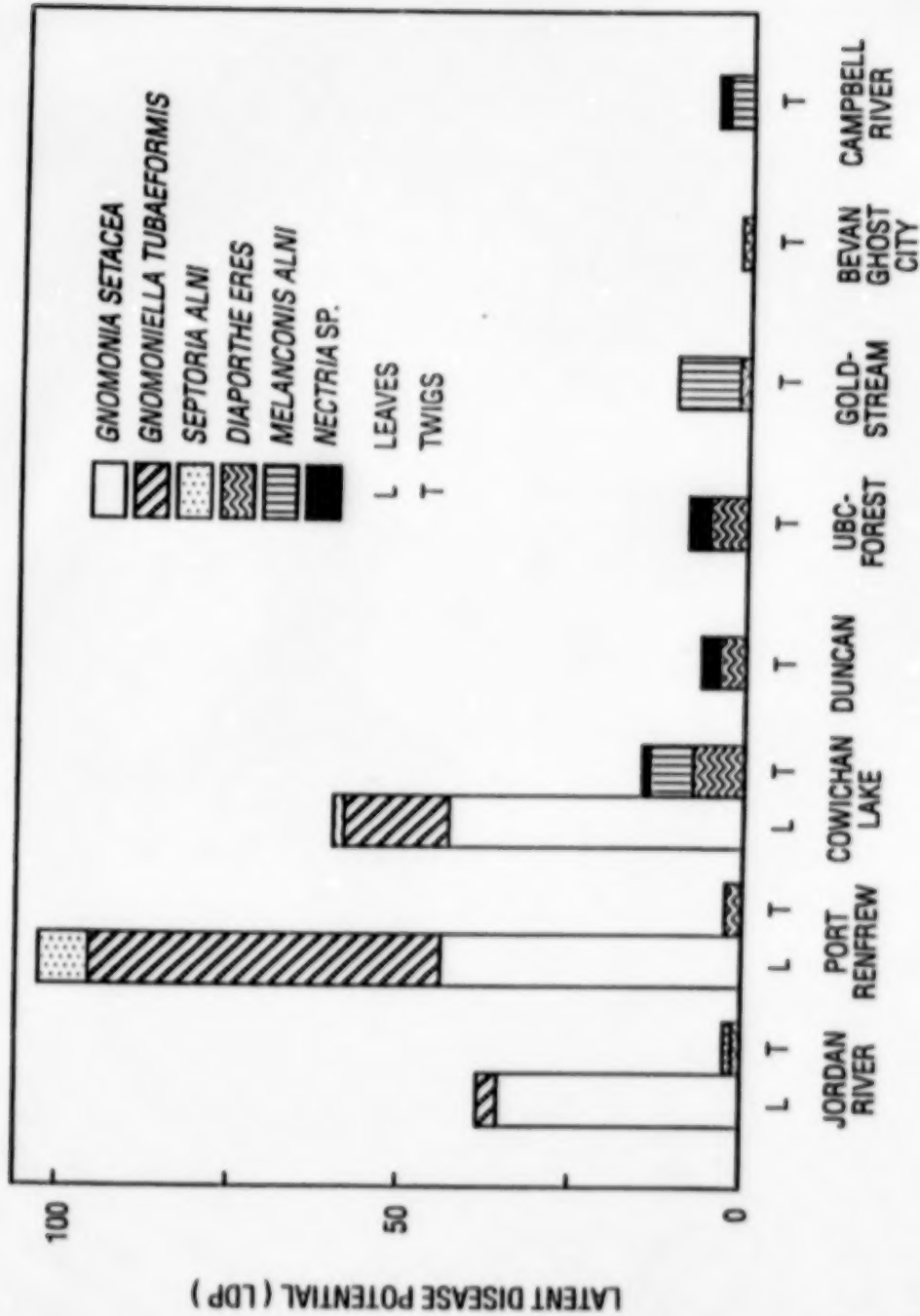


Fig. 10: Latent disease potential of red alder at each site.

This study showed once more that pathogenic fungi can be present in the host tissues even if there is no sign of disease. The plants are obviously able to constrain these fungi at least until conditions become unfavourable for the plant. The shift of the equilibrium in favour of the fungi may depend very much on the species and can be caused by changes of atmospheric conditions, by a weakened or senescent host, by activation of 'dormant' fungal genes, and possibly by alteration of the fungal genome.

In order to estimate the expected degree of diseases in case of a shift of the equilibrium in favour of the pathogenic endophytes the term 'LDP' (Latent Disease Potential) has been introduced. The LDPs depend very much on the site (and the pathogenicity of each endophyte). They could therefore be used to determine the site-specific composition and concentration of the mycoherbicide for the control of red alder and bigleaf maple.

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